AD678187

TRANSLATION NO. 2088

DATE: 11 January 1968

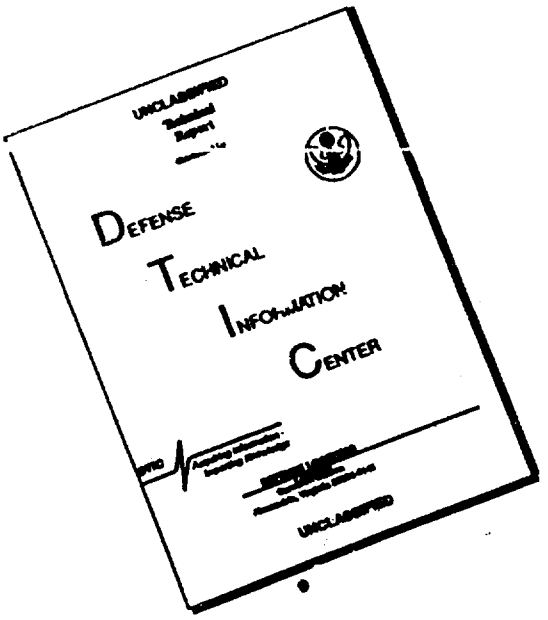
DDC AVAILABILITY NOTICE

Qualified requestors may obtain copies of this document from DDC.

This publication has been translated from the open literature and is available to the general public. Non-DOD agencies may purchase this publication from the Clearinghouse for Federal Scientific and Technical Information, U. S. Department of Commerce, Springfield, Va.

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

UDC: 576.858.9:576.851.511.

A COMPARATIVE STUDY OF ANTHRAX BACTERIOPHAGES

Zhurnal Mikrobiologii, Epidemiologii i Immunologii (Journal of Microbiology, Epidemiology and Immunology), No 9, 1967, pp 24-28.

Ye. N. Ievina and V. R. Arkhipova, Institute of Epidemiology and Microbiology imeni Gamaleya, Academy of Medical Sciences USSR, Moscow.

Among the various tests applicable for the purpose of identifying the causative agent of anthrax (capsule formation, sensitivity to penicillin, character of growth on culture media and others), the test of lysis by specific anthrax phages has gained in importance in recent years.

Anthrax phages have been known for more than 30 years (Rozgon; Gamaleya and others), but only in 1951 did McCloy obtain a highly active specific phage, having employed an original method of isolating it from lysogenic cultures of Bac. cereus and used an asporogenic culture of the anthrax microbe as an indicator strain. Since then various authors in different countries of the world, on using McCloy's method and strains as well as their own culture strains, obtained anthrax phages both from lysogenic cultures and from the soil (Brown and Cherry, 1955; Ivanovics and Lantos, 1958; Stamatin and Li Van Sob, 1959; Gruz, 1962; Meshcheryakov, 1962; Larina and Petrova, 1964 and others).

However, the authors indicated studied chiefly the specificity of action of the phages obtained by them on anthrax cultures and spore-bearing aerobes. Only isolated papers are known in which the study of the biological and antigenic properties of anthrax bacteriophages was carried out (Lantos and coauthors, 1960; Buck and coauthors, 1964; Zemtsova, 1965).

Thus, Lantos and coauthors (1960) studied the anthrax bacteriophages obtained by them from the soil in comparison with the McCloy phage and presented the first classification of anthrax bacteriophages. Buck and coauthors also carried out similar work (1963), but these phages proved to be of low activity as regards the antigen and this criterion, which is fundamental for classification, was not studied.

The task of the present article was the comparative determination of the biological antigenic and lytic properties of anthrax phages, both Soviet and foreign, which were in our collection, for the purpose of classification and determination of their specificity.

Six anthrax bacteriophages were subjected to study: BA-9 (Moldavian Institute of Epidemiology and Hygiene), Saratov (Saratov Mikrob Institute), Brown and Cherry γ bacteriophage (obtained from Prof. Seidel), McCloy αC and β bacteriophages (obtained from Prof. Ivanovics) and L_7 bacteriophage obtained from Prof. Stamatin).

The following properties of the bacteriophages have been studied: the morphology of the negative colonies, heat resistance, antigenic properties of the phages, and sensitivity of various strains of anthrax bacilli and spore-bearing aerobes to the bacteriophages studied. Moreover the preparation of optimal culture media for obtaining high-titer bacteriophages, the selection of indicator strains, the testing of various immunization systems for obtaining highly active antiphage serums, etc. entered into the research task.

The generally known methods of isolating pure lines and determining the heat resistance, the activity of antiphage serums, the adsorption of bacteriophages and the phagosensitivity of the strains described in textbooks on the bacteriophage (Adams, 1961; Gol'dfarb, 1961; Stent, 1965) are employed in the work.

The 1.25 and 0.7 percent agar prepared on the Hottinger digester, which contained 100 mg percent amino nitrogen, and broth with pH 7.2-7.4 prepared on the same basis proved to be optimum for obtaining stable forms of negative colonies of bacteriophages and high-titer bacteriophages.

Asporogenic and sporogenic strains, virulent and vaccine strains were used as indicator cultures; in this case the results conforming most to the pattern were obtained with the Davis strain (McCloy). It was necessary to renounce the use of sporogenic cultures, since negative colonies of these cultures were not successfully differentiated by morphology — they had various sizes and an indistinct configuration.

The bacteriophages with the highest titers, which contained $1 \cdot 10^{10}$-$1 \cdot 10^{11}$ corpuscles per ml, were obtained on solid culture media.

On studying the BA-9 bacteriophage on the Davis indicator strain three pure lines were isolated from the original phage. The phages of one line formed stable negative colonies similar in morphology to the δ phage — they were designated as BA-9$^{\delta}$ phages. The form of the negative colonies of phages of the second line was similar to the δ phage, but their size did not exceed one mm; the phages were unstable, constantly formed colonies similar to the BA-9$^{\delta}$ colonies, and were characterized by low activity — they contained up to $1 \cdot 10^{6}$-$1 \cdot 10^{7}$ corpuscles per ml. The phages of the third

line had the form of negative colonies with a clear lysis spot surrounded by a zone of incomplete lysis and with a diameter of up to three mm; they also proved to be unstable and formed colonies of the first two types.

A comparison of the morphology of negative colonies of anthrax bacteriophages permitted separating them into four groups (see figure): 1) the negative colonies were round in form, with smooth edges and a diameter of up to five mm, designated as c.l. phages (clear, large) and corresponded to the Ivanovich cg group; in this group are put the γ, BA-9$^{\gamma}$, Saratov, αC and Lη phages; 2) the negative colonies corresponded in form to phage colonies of the first group, but their diameter did not exceed one mm; this group is designated as c.s. (clear, small), they corresponded to the Ivanovich C.S.$^{\gamma}$ phages; the second line of the BA-9 phage is classed in this group and is called BA-9$^{c.s.}$; 3) the negative colonies up to three mm in diameter differed markedly from the colonies of the first two groups and consisted of a distinct center surrounded by a zone of incomplete lysis; this phage was named "clear with peripheral ring" after the form of the colonies; in this group is classed the third line of the BA-9 phage, which was named BA-9$^{inc.}$ and which corresponded to the Ivanovich Cr group; 4) the negative colonies were characterized by two forms, of which one corresponded to the form of the γ phage negative colonies and the other is known in the literature under the name bull's-eye colonies; under this type is classed the β phage, which consisted of mutants of the two phages and corresponded to the Ivanovich tlcg group.

Of the biological properties of bacteriophages the sensitivity to temperature and the rate of adsorption at different multiplicities were studied. All the bacteriophages proved to be highly sensitive up to 56°, except the αC phage (Table 1). They all possessed high adsorptive activity except the BA-9^{inc} phage (Table 2).

In studying the reaction of bacteriophage neutralization by antiphage serums an antigen kinship was ascertained between the γ and BA-9 phages which permitted combining them into a single antigen group (Table 3).

The antigen activity of the phages differed in the K value. The most active serums are obtained for the γ phage, the least active ones for the BA-9^{inc} phage.

The β, αC, Saratov and Lη phages can be classed in the other group of phages. These phages took part in the neutralization cross reaction with the Saratov and Lη antiphage serums. The β and αC phages proved to be low in activity for obtaining the corresponding antiphage serums.

The results obtained on the activity of antiphage serums differed from the data of Lantos and coauthors. These authors obtained the most active serums upon immunization by phages which corresponded in the morphology of the negative colonies to the BA-9^{inc} phage with a K value equal to 215-225, while in our experiments the serum obtained on immunization by this

TABLE 1

Thermal Inactivation of Various Bacteriophages at 56°

Inactivation time (in min.)	Index	Result of Thermal Inactivation of Phages						
		γ	BA-9$^{\gamma}$	BA-9$^{c.s.}$	BA-9^{inc}	β	αC	Saratov
15	Percent inactivation of bacteriophages	95.2	100	99.5	97.7	94	70	97
30		98.4	100	99.4	98.5	96	95	97
15	Inactivation rate constant (in min^{-1})	0.79	0.76	0.71	0.55	0.78	0.35	0.41

TABLE 2

Adsorption of Various Bacteriophages on the Davis Strain

Phage	Number of corpuscles per ml.	Multiplicity	Percent adsorption in various time periods (in min.)		
			10	20	30
γ	$6.8 \cdot 10^{10}$	0.68	98.3	98.9	99.7
BA-9$^{\gamma}$	$1.6 \cdot 10^{10}$	1.6	95	96	96
BA-9$^{c.s.}$	$1.1 \cdot 10^{8}$	0.1		80	80
BA-9^{inc}	$1.5 \cdot 10^{9}$	0.3	0	0	0
β	$1.79 \cdot 10^{11}$	0.17	97	98.9	98.9
αC	$5.3 \cdot 10^{10}$	0.5	95.5	96	97.6
Saratov	$5.3 \cdot 10^{10}$	2.4	99.2	99.5	97

phage is less active than for the phages similar to the γ phage in morphology (K = 27 and 310 respectively).

In phages having an identical morphology of the negative colonies, such as for example γ and Saratov, the serological cross reaction could not be observed, which probably testified to differences in the antigenic properties of these phages.

For the purpose of testing the lytic properties 50 anthrax strains of different virulences, which were isolated from the soil and from hides and obtained from a museum, and also 85 strains of spore-forming aerobes

TABLE 3

Neutralization Cross Reaction of Bacteriophages with Antiphage Serums
(Neutralization Rate Constant)

Serums 1:100 \ Phages	γ	BA-9γ	BA-9c.s.	BA-9inc	β	αC	Saratov	L7
	310.0	148	176.6	136	0	0	0	0
BA-9	129.7	58	112.8	66	0	0	0	0
BA-9inc	127.5	61.9	75.5	27	0	0	0	0
Saratov	0	0	0	0	189	164	262	151
L7	0	0	0	0	252	49	156	146

were studied. The γ and BA-9γ phages (Table 4) had the widest spectrum of lytic activity. Under the action of the BA-9inc phage lysis with a thin semi-transparent film of bacteria growth was noted. The remaining bacteriophages — β, αC and Saratov — did not cause lysis of from 14 to 22 percent of the anthrax strains. Moreover, these phages did not bring about non-specific lysis of the strains from the spore-bearing aerobe group, while under the influence of the γ and BA-9 phages from 3 to 8 percent non-specific reactions were observed.

TABLE 4

Results of Phagosensitivity of Bac. anthracis and Bac. cereus to Various Phages

Microbe species	Total no. of cultures	Behavior toward phages	γ	BA-9	BA-9c.s.	BA-9inc	β	αC	Saratov
Bac. anthracis	50	Sensitive	49	50	49	49	39	39	43
		Insensitive	1	0	1	1	11	11	7
Bac. cereus	83	Sensitive	4	6	3	7	1	1	1
		Insensitive	79	77	80	76	82	82	82

CONCLUSIONS

1. A study of the morphology of negative colonies of various anthrax bacteriophages showed that according to morphology the negative colonies of anthrax bacteriophages included all the known types described in other bacteriophages.

2. On the basis of the study of the antigenic properties the anthrax bacteriophages can be divided into two groups: the γ (Brown and Cherry) and BA-9 (Moldavian Institute of Epidemiology and Hygiene) bacteriophages should be classed in one group; in the other group — the αC, β (McCloy), Lη (Stamatin) and Saratov bacteriophages.

3. Separation of the anthrax bacteriophages studied into two groups corresponded to their lytic properties. Phages belonging to the first group had a wider lytic spectrum, not only with respect to homologous microorganisms but also with respect to Bac. cereus.

4. For the purpose of increasing the specificity of the phagolysis test for identification of anthrax cultures one should at present simultaneously use anthrax bacteriophages belonging to different antigen groups, for example BA-9γ and Saratov, or γ and αC bacteriophages.

BIBLIOGRAPHY

1. N. F. Gamaleya, Biologicheskiye Protsessy Razrusheniya Bakteriy (Biological Processes of Disintegration of Bacteria), Moscow-Leningrad, 1954.

2. D. M. Gol'dfarb, Bakteriofagiya (Bacteriophagy), Moscow-Leningrad, 1961.

3. Ye. V. Gruz, in the book Antigeny Mikroorganizmov i Otvetnyye Reaktsii (Antigens of Microorganisms and Reciprocal Reactions), Kishinev, 1962, page 127.

4. I. M. Zemtsova, Kharakteristika Sibireyazvennogo Bakteriofaga i yego Primeneniye dlya Laboratornoy Diagnostiki Sibirskoy Yazvy (Characteristics of the Anthrax Bacteriophage and its Utilization for Laboratory Diagnosis of Anthrax), Candidate's Dissertation, Saratov, 1965.

5. V. S. Larina and L. S. Petrova, in the book Mikrobiologiya i Immunologiya Osobo Opasnykh Infektsiy (Microbiology and Immunology of Especially Dangerous Infections), Saratov, 1964, page 94.

6. A. Ya. Meshcheryakov, Trudy Vsesoyuznogo Instituta Eksperimental'noy Veterinarii (Transactions of the All-Union Institute of Experimental Veterinary Science), Vol 26, 1962, page 46.

7. F. N. Rozgon, Veterinarnoye Delo (Veterinary Science), No 7(68), 1929, page 14.

8. M. Adams, Bakteriofagi (Bacteriophages), Moscow, 1961.

9. E. R. Brown and W. B. Cherry, J. Infect. Dis., Vol 96, 1955, page 34.

10. C. A. Buck, R. L. Anacker, F. S. Newman et al., J. Bact., Vol 85, 1963, page 1423.

11. G. Ivanovics and J. Lantos, Acta microbiol., Acad. Sci. hung. Vol 5, 1958, page 405.

12. J. Lantos, J. Varga, and G. Ivanovics, Ibid., Vol 7, 1960, page 31.

13. E. W. McCloy, J. Hyg., Vol 49, London, 1951, page 114.

14. N. Stamatin and others, Arch. roum. Path. exp., Vol 18, 1959, page 31.

Submitted to the editor on 28 September 1966.